**3136-Plat**

**Two-Photon Scanned Light Sheet Microscopy Reveals Spatio-Temporal Organization of Cells and Proteins in Developing Embryos**

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Light sheet microscopy, with its novel sheet-illumination orthogonal to the detection direction, has been shown in recent years to achieve higher acquisition speed and lower photodamage than conventional techniques for in vivo imaging of cells and organisms. We recently applied 2-photon excitation to light sheet microscopy to increase the penetration depth, allowing long-term imaging of cells deep inside of live embryos. Here we show the application of this novel imaging technology to record the cellular and protein dynamics in developing embryos. The combination of subcellular spatial resolution, high acquisition speed, and high penetration depth allows study of the spatio-temporal organization and control of cells and proteins that are critical in the development of an organism.

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**Fluorescence Correlation Spectroscopy Imaging of Chromatin Dynamics**

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Fluorescence correlation spectroscopy (FCS) is a confocal microscopy-based method allowing to assess diffusion, transport and interaction properties of molecules (proteins, nucleic acids, compounds) in vitro and in vivo. Commercially available instruments enable routine measurements at one or few specific points inside living cells.

FCS experiments inside living cells or embryos remain a challenge since point measurements often feature large errors caused by the heterogeneous environment of the sample. Moreover, biological noise due to cell-to-cell differences of physical and biological parameters (viscosity, protein expression) induces further variations that are difficult to separate from measurement error. Currently, these problems are addressed by performing statistical data analysis of measurements from many cells. To significantly improve the method, FCS measurements can be conducted simultaneously in many points per cell.

Here we present a recently introduced microscopy setup [1] that allows spatially resolved FCS measurements in 2D optical sections across cells. The setup is based on a thin diffraction-limited light sheet that illuminates a cross-section of the cell. An EM-CCD camera placed perpendicular to the light sheet provides thousands of point detectors and enables to record in each pixel the incoming photons with single photon sensitivity, sub-millisecond time resolution and close-to-confocal spatial resolution.

We have used this and a confocal setup to perform measurements of the diffusion- and binding-related mobility of chromatin-forming and -associated GFP-labeled proteins inside nuclei of living cells in interphase. We could identify and characterize the binding of heterochromatin protein 1 and of histone proteins to chromatin as well as the local dynamics of the chromatin fiber in different nuclear localisations, supporting the existence of subchromosomal domains with distinct properties.

[1] Capoulade, J., Wachsmuth, M., Hufnagel, L. & Knop, M. Nat. Biotechnol. 29 (2011), 835-839.

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**In Vivo Metabolic Mapping of Stem Cells and Differentiated Progeny in Small Intestine and Colon Crypts by Phasor Fluorescence Lifetime Microscopy**

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We have performed label-free Phasor Fluorescence lifetime microscopy (FLIM) to reconstruct the three dimensional metabolic signature of small intestine and colon tissue *in vivo*. Our method provides a label-free identification and metabolic mapping of stem cells during differentiation. Freshly excised tissues are imaged with two photon microscopy and FLIM within two hours. Lgr5-GFP mice are used to mark the Lgr5+ stem cell population at the base of small intestine (SI) and colon crypts. Using Phasor FLIM analysis of live tissue excited at 880nm and 740nm, we identify and map the concentration of different intrinsic metabolic fluorophores and extracellular matrix elements such as NADH, FAD, and collagen. We observe that different compartments of the tissue are defined by unique Phasor FLIM signatures. We can distinguish collagen fibers at the base of the crypts, the lamina propria, the vascular network and the epithelium. The FLIM signature at the base of the crypt at 740nm follows exactly the map of stem cells intercalated between adjacent Paneth cells. Paneth cells are characterized by a different FLIM signature with respect to the stem cells thus indicating a difference in the concentration and/or composition of intrinsic fluorophores. The FLIM Z-stack reveals a shift of the metabolic signature of crypt epithelial cells during differentiation. Stem cells at the base of the crypt have the shortest lifetime and the highest NADH/ NAD+ ratio. Movement up the crypt to transit amplifying cells and fully differentiated cells on the mucosal surface corresponds to different FLIM signatures that correspond to decreasing NADH/ NAD+ ratios, as is expected during differentiation.

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**Tracking Image Cross-Correlation for Elucidating the Fusion Process of Viruses**

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Promising as a gene carrier, the Foamy virus (FV) is an atypical retrovirus that shares similarities with HIV and the hepatitis B virus. Despite numerous biochemical studies, its entry pathway remains unclear, namely whether entry occurs through fusion at the plasma membrane or after endocytosis. To investigate this issue, dual-color fluorescent viruses were engineered with a GFP-labeled capsid and a mCherry-labeled envelope. Thus, the release of the capsid from the envelope would result in a loss of colocalization of the two colors at the single virus level. Using 3D single virus tracing, we followed the entry of the fluorescent viruses in living cells. In such experiments, a compromise is made between low phototoxicity for the cells and sufficient signal-to-noise ratio for tracking. Therefore, it is often difficult to rely on the fluorescence intensity to estimate colocalization. Cross-correlation approaches are highly sensitive to colocalization and coordinated motion but none of the existing methods are applicable to single particle tracking. We developed a novel approach, Tracking Image Cross-correlation (TrIC), which combines image cross-correlation and single particle tracking. The image cross-correlation analysis yields the colocalization status of the particle as well as the average distance between the two labels. The combination of this dynamical colocalization information with the instantaneous velocity of the particle and its position within the cell allows us to clarify the Foamy virus entry pathway. We compared two types of FVs and demonstrated that the prototype FV can enter the cell by either endocytosis or membrane fusion whereas the simian FV was only observed to fuse after endocytosis. Interestingly, our analysis revealed an intermediate stage during the fusion process where the capsid and envelope separate by ~400nm but are still attached for ~5 minutes before fusion is completed.